Excitation, Contraction, Coupling

Introduction

During contraction, actin and myosin filaments slide past one another. They do this by a series of processes that involves exposing myosin-binding sites on actin, using myosin binding to generate a powerstroke, and then using ATP to reset the process. The powerstroke occurs nonsimultaneously—each myosin head acts independently. Contraction is initiated by Ca^{2+} entry into the cell. Calcium binds to troponins. Troponin/tropomyosin moves out of the way. Myosin heads bind to actin. Powerstroke occurs. Let's explore the details of these processes.

Action Potential to Calcium

The plasma membrane (called the sarcolemma in a muscle) surrounds an entire muscle fiber (a single muscle cell). The fiber is made of fibrils lined up next to each other. Each **fibril** is surrounded by a **sarcoplasmic reticulum**. The sarcoplasmic reticulum **stores Ca^{2+}**.

When the presynaptic neuron activates, an action potential is propagated to the presynaptic terminal. In skeletal muscle, one neuron innervates multiple fibers. The neuron along with the fibers it innervates is called a **motor unit.** The ACh released induces nicotinic ACh-receptor activation, opening Na^+/K^+ channels at the endplate of the multiple fibers, inducing an action potential in the cell membrane of multiple fibers.

In each fiber, that action potential travels along the plasma membrane (sarcolemma) of the muscle fiber. At reliable intervals, the plasma membrane invaginates into the cell to pass near fibrils. These reliable intervals occur always at the **A-band–I-band junction.** The sarcoplasmic reticulum membrane rests on the cell membrane's invagination. These tubular projections of the cell membrane are called transverse tubules (**T tubules**). Each T tubule travels through the cell, past the A-I junction, with sarcoplasmic reticulum on either side. This combination of T tubule with two sarcoplasmic reticula is referred to as a **triad**.

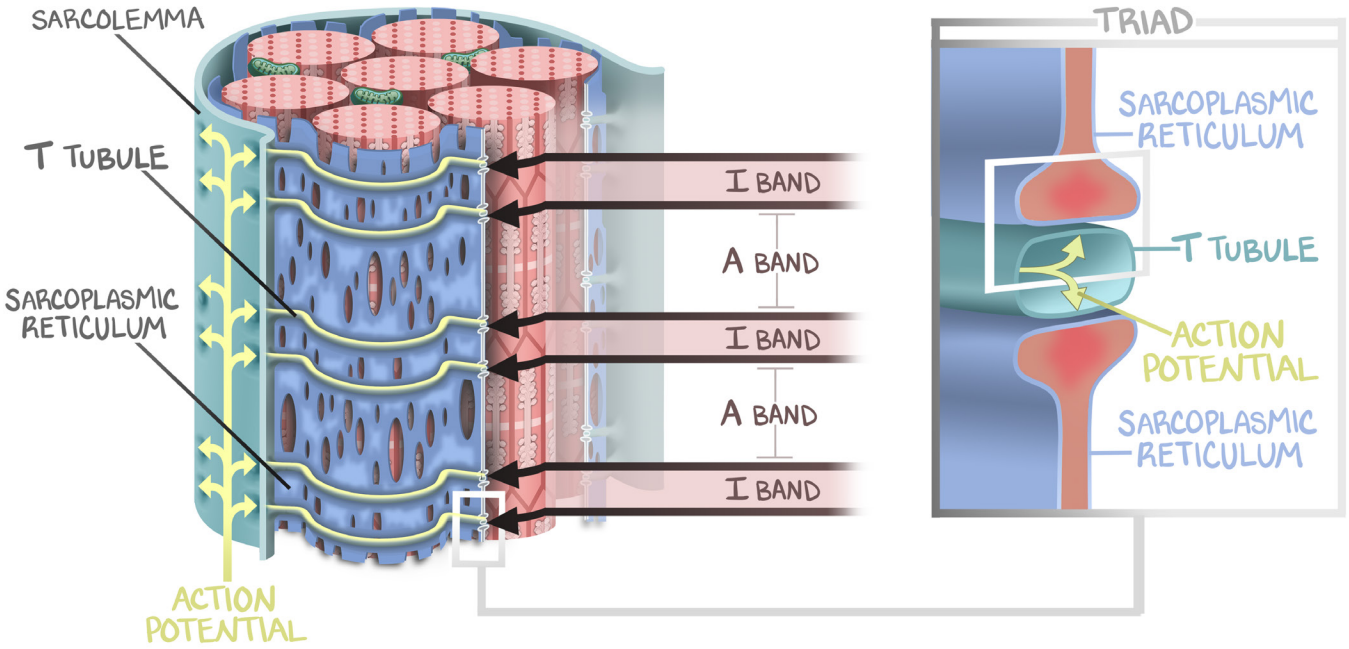

Figure 12.1: Membranes and Triads
The sarcolemma periodically invaginates, sending T tubules through the cell across myofibrils. Sarcoplasmic reticula follow very closely for the length of the T tubule. The three tubes running together—sarcoplasmic reticulum, T tubule, sarcoplasmic reticulum—is called a triad. Triads always penetrate at the A-I band junction. [As the action potential from the endplate is propagated down the sarcolemma, down the T tubule, voltage-gated calcium channels open. Those calcium channels opening on the T tubule are physically linked to the mechanical gate on the membrane of the sarcoplasmic reticulum. Moving the gate opens the gate, dumping calcium into the cytoplasm.]

The action potential propagates through the T tubule. That depolarization activates and opens the gate of **T-tubule voltage-gated L-type calcium channels**. These channels on their own do not allow for sufficient calcium movement to cause contraction, because they aren't in the sarcoplasmic reticulum. However, they are **mechanically coupled** with a different calcium channel that's in the sarcoplasmic reticulum membrane. As these T-tubule L-type Ca^{2+} channels open, they physically (mechanically) open sarcoplasmic-reticulum-**ryanodine-Ca^{2+} channels**. A massive store of calcium present in the sarcoplasmic reticulum dumps into the cytoplasm. It's THIS calcium influx that is sufficient to induce contraction.

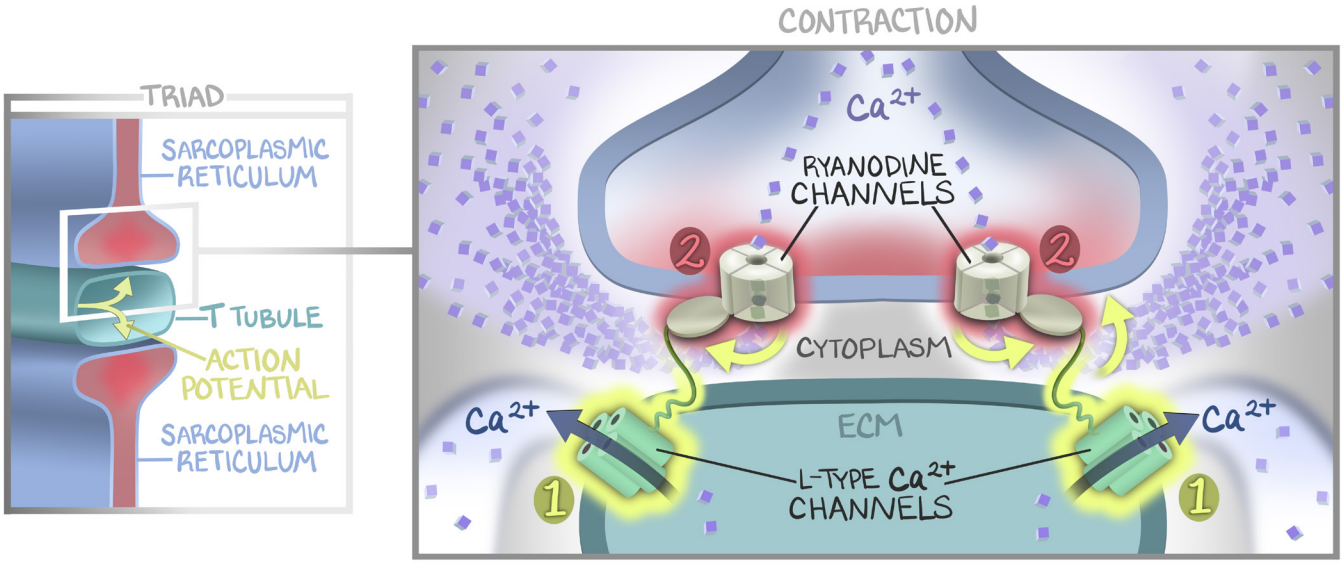

Figure 12.2: Triads and Calcium Channels
The sarcolemma periodically invaginates, sending T tubules through the cell across myofibrils. Sarcoplasmic reticula follow very closely for the length of the T tubule. The three tubes running together—sarcoplasmic reticulum, T tubule, sarcoplasmic reticulum—is called a triad. Triads always penetrate at the A-I band junction. As the action potential from the endplate is propagated down the sarcolemma, down the T tubule, voltage-gated calcium channels open. Those calcium channels opening on the T tubule are physically linked to the mechanical gate on the membrane of the sarcoplasmic reticulum. Moving the gate opens the gate, dumping calcium into the cytoplasm.

Calcium and Thin Filaments

Thin filaments are F-actin. F-actin is made up of discrete globular actin proteins called G-actin. F-actin polymers are surrounded by and inhibited by the troponin-tropomyosin complex. **Troponin I** ("inhibitory troponin") is physically in the way, covering the myosin-biding site on actin, preventing association of myosin to actin. **Troponin T** (TnT) pushes the tropomyosin aside, inducing the conformational change in the troponin/tropomyosin complex that exposes the myosin binding site. Calcium binds to **troponin C** ("calcium-binding troponin"). When calcium enters the cytoplasm (where the sarcomeres are), it binds to TnC, inducing TnT to undergo a conformational change, rolling back on the actin filament, lifting TnI away to reveal the myosin-binding site. This allows the myosin chain to bind to the actin filaments to initiate a powerstroke.

Calcium plays the role of **revealing the myosin-binding sites on actin**. The more calcium present, the more TnC is bound to calcium. This in turn means more TnT moves TnI out of the way, and therefore more myosin heads can bind at once. More calcium, more contraction.

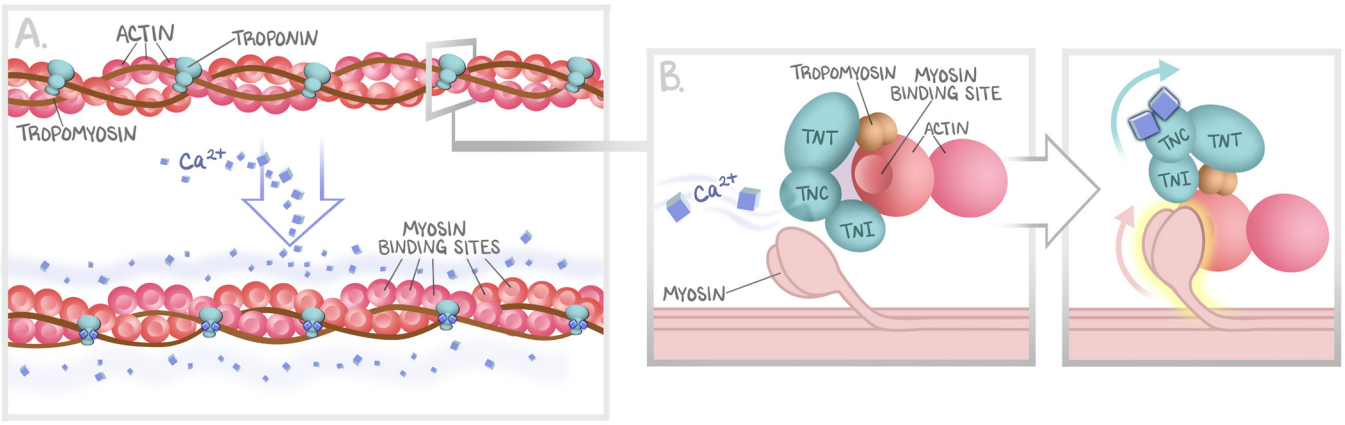

Figure 12.3: Calcium Trop Crossbridge
(a) Demonstration of the actin filament and the consequence of calcium binding—exposure of myosin-binding sites on F-actin. (b) Local effect of one myosin heavy chain interaction with one G-actin molecule. Calcium binding to TnC causes a conformational change in TnT, revealing a myosin-binding domain, previously covered by TnI, allowing for cross-bridge formation. If the domains are not revealed, it doesn't matter what conformation the myosin is: no powerstroke can be initiated because myosin-binding sites are unavailable on actin.

Cross-Bridge Cycling

Now that you understand calcium and actin, let's discuss myosin and ATP.

Contraction of muscle requires the **entrance of calcium** from the sarcoplasmic reticulum to reveal the myosin-binding sites on actin by moving tropomyosin, AND the **presence of ATP** to both release myosin from its finished-powerstroke position and provide energy for the conformation change for the next powerstroke.

Myosin starts attached to a myosin-binding site on actin. In this state, it has neither ATP nor ADP. The addition of **ATP to myosin** causes it to **relax** and **dissociate from actin**—this places it in the **released state**. Myosin heads hydrolyze ATP to ADP and phosphate, but **retain both**, resulting in a **cocked state.** In this cocked state, the myosin head is now ready to grab hold of actin. If a myosin-binding site is available, myosin will latch onto actin. No conformational change has occurred to myosin, but, attached to the actin, it's now said to be in the **cross-bridge state**. Myosin is then dephosphorylated, resulting in the **powerstroke**. ADP is also cleft from myosin, resulting in the original **attached state**.

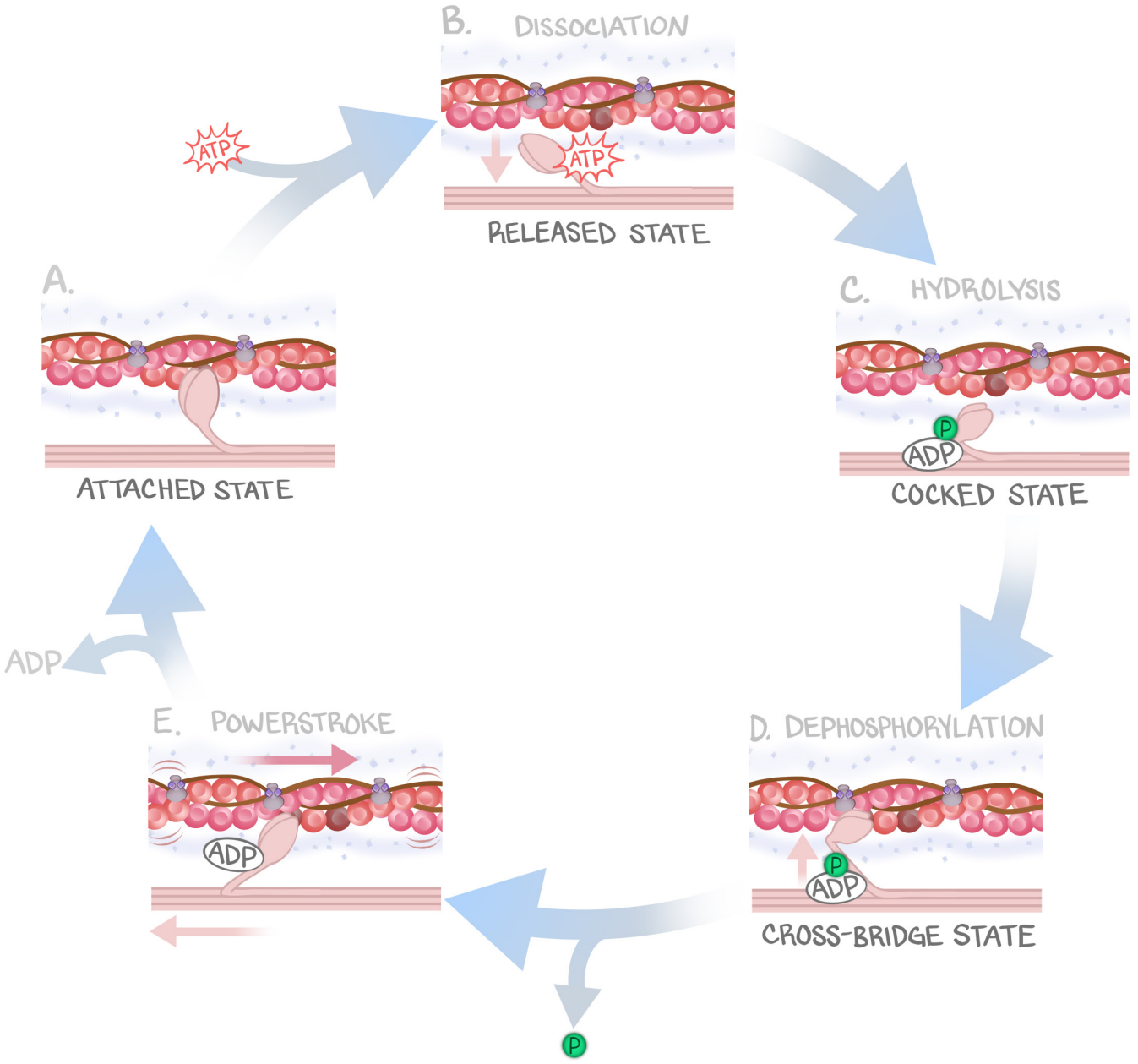

Figure 12.4: Cross-Bridge Cycling
This is a cycle; we begin at the attached state arbitrarily. (a) In the attached state, myosin has no ATP or ADP. (b) ATP causes dissociation of the myosin heads. (c) Innate hydrolysis of ATP by myosin places myosin in the cocked state. (d) If myosin-binding sites are available, myosin binds to actin, and the powerstroke occurs. After powerstroke, ADP is released, and here the myosin stays until ATP dissociates myosin from actin again.

Rather than regulate ATP utilization, muscle cells regulate available myosin-binding domains on actin. Without a binding site to latch onto, there would be no dissociation of phosphate, and the myosin head would remain in the cocked position. This explains rigor mortis. When a patient dies, the body's cells run out of oxygen. No oxygen, no ATP. Myosin heads remain in the attached state (the "stroked" state) and cannot relax or dissociate from actin. This inability to relax is what causes the stiffness of muscles in rigor mortis.

Relaxation

The action potential lasts only 2 milliseconds, but the contraction can last for 100 ms. The cessation of contraction, therefore, cannot correlate with the cessation of the action potential. Calcium is the real driving mechanism of contraction, so it's the sequestration of calcium that causes contraction to cease. Contraction is about getting the calcium out of the sarcoplasmic reticulum and into the cytoplasm. **Relaxation** is about getting the **calcium back into the sarcoplasmic reticulum.**

Sarco/endoplasmic reticulum Ca-ATPases (**SERCAs)** are ATP-driven active pumps that exchange H^+ for Ca^{2+}. They work at the plasma membrane (the T tubule), removing calcium into the extracellular matrix, and they work on the sarcoplasmic reticulum. Calcium-binding proteins, particularly **calsequestrin**, bind and stabilize calcium in the sarcoplasmic reticulum. The calcium sent to the sarcoplasmic reticulum will be available for the next contraction. Calcium released into the extracellular matrix will not.

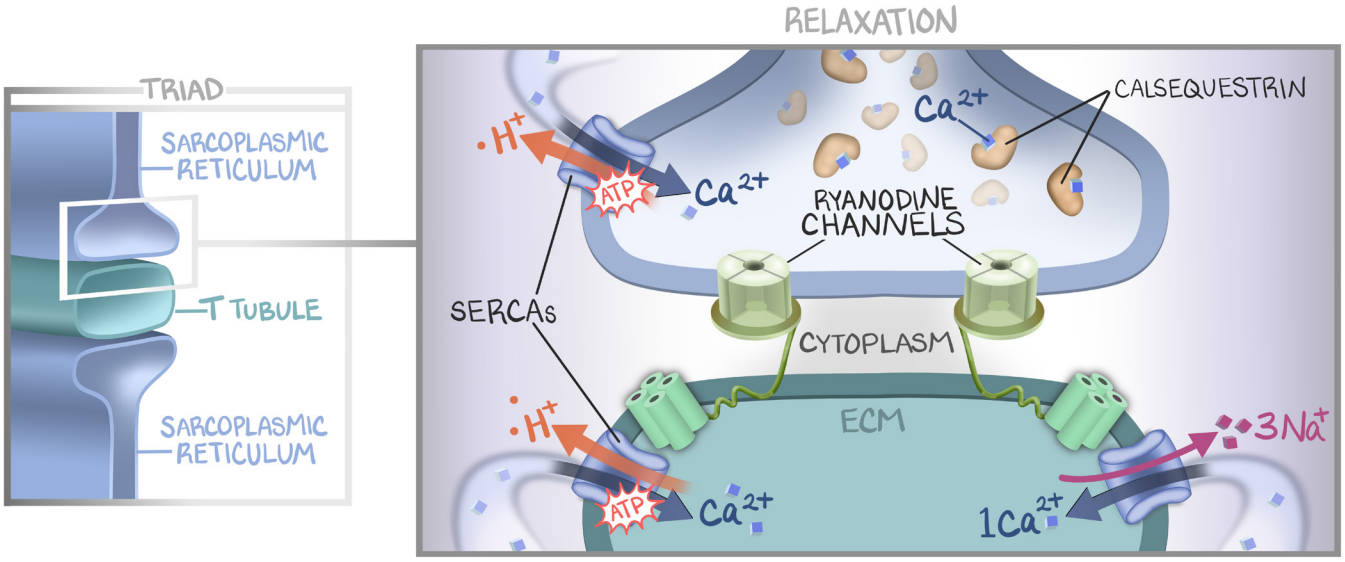

Figure 12.5: Calcium Sequestration and Relaxation
To terminate the signal for contraction, skeletal muscle regulates cytoplasmic calcium. SERCAs are ATPase H^+-Ca^{2+} antiporters and are located on the sarcoplasmic reticulum and the sarcolemma. Na^+-Ca^{2+} antiporters (harnessing the natural concentration gradient of Na^+) are located only on the sarcolemma. Once inside the sarcolemma, calsequestrin stabilizes Ca^{2+}.

In addition to SERCAs, the **plasma membrane** has a **Na^+-Ca^{2+} antiporter**. Taking advantage of Na^+'s concentration gradient, 3 sodium ions are allowed into the cell, trading it for 1 calcium extruded into the extracellular matrix. Because it's able to use the Na^+ concentration gradient, it requires no ATP—the antiporter itself is ATP-independent. But that concentration gradient is established by the Na^+/K^+-ATPase, which does require ATP.